THE REVERSAL OF LETHALS IN DROSOPHILA

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PREFACE

The author wishes to express deep appreciation to both Dr. J. T. Patterson, who directed this work, and Dr. W. S. Stone for their constructive comment and criticism during the course of this work. It has proved to be of inestimable value during the whole course of the experiments. Appreciation is also due Mr. A. B. Griffen for his unselfish help in the preparation and interpretation of the cytological material

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INTRODUCTION

It seems quite probable that lethal mutations have been employed more frequently in recent years than almost any other type of mutation among genetic phenomena. In practically all of these cases, however, the interest has centered almost entirely upon the mutation process and the mutation rate rather than upon the nature of the mutants themselves. Lethals lend themselves readily to this type of study, since it is only with them that one is able to deal objectively and quantitatively with the mutation rate. The assumption in the case of all of these experiments has been that there is no fundamental difference in the nature of a lethal mutation and a visible mutation; the only apparent difference is that the former is "physiological" while the latter is primarily "morphological" (at least from the point of view of the average geneticist.) There is, of course, no reason for believing that the two categories are mutually exclusive, since the lethal qualities of a mutant might conceivably depend upon some morphological abnormality.

This view which is held by many is well summarized by Muller (1938 a). In discussing the works of Timofeeff-Ressovsky and others on mutations that are not fully lethal, the idea is expressed that there is a gradation from the fully viable mutants through a " --- not unlikely --- still larger class of mutations that have effects too slight or too hidden to have yet been demonstrable." Next, there is a class of semi-lethals, such as those studied by Timofeeff-Ressovsky, and finally the smaller class of fully lethal mutations. That is to say, there is no absolute line of demarcation separating a "lethal" from a "non-lethal" mutation.

The contrary view is taken by Demerec (1936 a) who states that "Data are also accumulating to indicate that a great majority of lethal factors are deficiencies." His evidence for this statement consists of a cytological study of some thirty lethals in which he reports that every case is a deficiency as seen in the salivary chromosomes. In another paper (Demerec 1936 b) he states that, in reference to random lethals, "Probably all or at least a great majority of them are deficiencies."

It is of interest to note the work of Painter and Patterson (1935) in the mapping of the third chromosome of D. melanogaster. They found 22 cases which were lethal in the homozygous condition. Of these there were 7 deletions,

3 translocations, 3 inversions, and 9 cases where absolutely no rearrangement could be detected. This would indicate either rearrangements too small to be seen, or point lethals. More recently there has appeared some work by Sacharov (1938) in which he finds only one deletion in 112 lethals, all the others proving to be point mutations.

Recent work by Stadler and associates (Stadler and Sprague 1936, Stadler and Uber, and Stadler 1937) is of interest here, since by the use of monochromatic ultra-violet light of different wave lengths they show the separability of various genetic effects of irradiation. Certain wave lengths will produce deficiency and mutation, but not translocation; other wave lengths produce no deficiencies, but produce germless seed, which are interpreted as the effect of "dominant lethals" which prevent the normal embryo formation. Stadler states however that there is no proof that this type of change is genic or even chromosomal.

Another indication of the fundamental difference between various types of genetic changes is given by Muller (1938 b), who points out that while mutations and minute rearrangements vary directly with the dosage, translocations appear with disproportionate frequency with increases in the dosage of X-rays.

Another aspect of the lethal problem is its relationship to the question of position effect. Dobzhansky (1936) points out that the frequent lethal effect of chromosomal

abnormalities (other than deletions) may be explained by one of three means: (1) position effect, (2) simultaneous breakage and point mutation, or (3) loss of genic material at the point of breakage.

Because of the present situation it should be of fundamental importance to determine the exact nature of lethals, since it is an important phase of the work on the mutation process. That is, according to an idea of random mutation, one would expect the production of mutations of varying harmfulness, a s stated by Muller in the article cited above (1938 b).

The most proper approach to this problem appears to be the usage of reverse mutation as a test of the presence or absence of the affected area of the chromosome. This is a classical method, used by Patterson and Muller (1930) on the genes scute and forked. Their work showed that X-ray mutations were not produced by a loss, for if that were the case mutations in the reverse direction could not be produced. Timofeeff-Ressovsky (1930) did considerable work on reverse mutations, using numerous loci in both the X chromosome and in chromosome 3. These works were followed by the work of Johnston and Winchester (1934) who obtained quite a few reverse mutations of numerous X chromosome mutants.

At the present time there have been only two minor publications on the subject of lethal reversal. One of these

(Suche, et al 1938) reported the preliminary work of several related experiments, including the present study.

Oliver (1938) in studying the 2-3 translocation Punch discovered a reversal of the eye-color which also affected the lethal properties of the translocation. The reversed lethal was not viable in the homozygous condition, but was perfectly viable when combined with the original lethal. No cytological observations were made, but genetic tests (linkage of Cy and D) showed the translocation to be still present. This did not preclude the possibility of a more involved chromosome rearrangement, however.

EXPERIMENTAL METHODS

It was decided to study lethals from the standpoint of both "position effect" lethals and point lethals. For the former several 2-4 translocations produced in this laboratory by Patterson, et al (1934) were chosen. They are as follows: T2,4-A 1, T2,4-A 12, and T2,4-A 45, all of which are lethal when homozygous. These stocks were carried using Curly as a balancer, so that an additional position effect lethal was being simultaneously irradiated. These stocks were chosen for the experiment because all have breakage points near enough to those of the Cy L inversion that crossing-over is well nigh impossible.

The point lethals used in this experiment were produced especially for that purpose from X-rayed wild-type females.

Two such second chromosome lethals, lethal-1 and lethal-7, were chosen for the study; a thorough cytological examination in the salivary chromosomes failed to reveal the slightest abnormality in either case. These stocks, like the translocations, were carried balanced to Curly.

The technique used in practically all of the cases was to X-ray the males and cross them to their virgin sisters. After not over five days the parents were discarded, so that the offspring were derived from the irradiated haploid sperm, thereby eliminating the possibility of duplication of "lost" portions. The irradiation used was approximately 4000 R units.

In one case (T2,4-A 12) a small group of females were X-rayed and crossed to stock males. The technique of handling after this was essentially the same as for the males. The irradiation used was approximately 2000 R units. One female non-Curly fly was produced from this portion of the experiment; details are to be found in the section of the paper dealing with experimental results.

Lethal reversals in such an experiment will be detected by the appearance of a non-Curly fly. Such flies of this type as appeared were bred and their offspring subsequently examined cytologically to determine what changes had taken place. In most cases the method was to cross a non-Curly male, heterozygous for the reversal, to wild-type virgin females. By examination of the larvae it was possible to

determine in which chromosome the change had taken place. In case of a reversal of the Curly chromosome, one half of the offspring would show the Curly inversions in whole or in part. In case of a reversal in the point lethal or the translocation chromosome, there should be no trace of the Curly inversions.

In some of the cases (lethal-1 reversals numbers 1, 2, and 5) the cytological observations were made on the offspring from a cross of a non-Curly female, heterozygous for the reversal, to stock males (lethal-1/Cy). In these cases if there was a reversal of the Curly character with no change in the lethal, all of the offspring should show the typical Curly inversion figure. In case of a reversal of the point lethal or the Curly lethal, there should be some larvae in which both homologues of chromosome 2 would be perfectly paired. By comparison with the cytological map of the normal second chromosome it would be possible to determine whether the lethal-1 or the Curly chromosome has been affected.

Figure 1 shows a typical Curly inversion figure. (This chromosome was drawn from case T2, 4-A 45 R-4).

EXPERIMENTAL RESULTS

Out of an approximate 100,000 F_1 flies examined there were produced 18 fertile non-Curly individuals.

Several types of reversals are to be found in this group: (1) reversals of Curly, (2) reversals of translocation lethals, and (3) reversals of a point lethal. Each reversal stock is labeled with the stock number and the number of the reversal of that particular stock. For example, the first reversal picked up in the stock T2,4-A 12 is lateled T2,4-A 12 R-1, and so forth.

A. Translocation-lethal reversals

1. T2,4-A 12 R-1 arose from a non-Curly female which bred giving non-Curly offspring. This stock was kept in mass culture for several generations, then examined cytologically by crossing the non-Curly males to wild type virgin females. Fourteen larvae from this cross were examined and all showed the translocation in the salivary chromosomes. In one case in addition to the translocation, the Curly inversion in the right arm of the second chromosome was present. This may have occurred by crossing-over in the stock at some time during the course of the experiment, or after the reversal was picked up. There was no noticeable change in the translocation figure in any of the cases examined.

2. T2,4-A 1 R-7. It is impossible to say for sure exactly what the situation here is. Apparently it is a reversal of the translocation lethal, since the offspring from a non-Curly male, heterozygous for the translocation, and the reversal, crossed to wild virgin females showed no Curly inversions in the salivary chromosomes. Only 8

larvae were examined; in 6 of these it was possible to see the translocation figure. In the other two it was impossible to tell whether a translocation was present or only normal chromosomes. Further tests will have to be made to clear up this point. Tentatively, however, the case will be put under the heading of a reversal of a translocation lethal.

B. Curly reversals

1. lethal-7 R-1 males, non-Curly, were crossed to wild type virgin females and the salivary chromosomes of the larvae examined. The figures from these showed either a regular Curly inversion figure or a perfectly normal second chromosome, showing that the change had taken place in the Curly chromosome rather than in the lethal-7 chromosome. This Curly reversal is still lethal, however, since the gene purple which is present in the Curly stock never became homozygous.

2. lethal-7 R-2 males, non-Curly, were crossed to wild type virgin females, and several types of figures were found in the salivary chromosomes of the progeny. Some showed the complete Curly inversion figure, while others had only the Cy 2L or 2R inversion with the other arm normal. Still other larvae had completely normal salivary chromosomes. Separation of the two Curly inversions is undoubtedly due to crossing-over. This reversal like

the former does not live in the homozygous condition, apparently.

3. T2,4-Al R-1 is a very interesting case, since it arose spontaneously in the stock rather than from the X-rayed animals. A female with one Curly wing and one normal wing was crossed to her brothers, and a few non-Curly flies appeared in the next generation. Subsequently males from this stock were crossed to wild virgins and the salivary chromosomes examined. Some of the slides showed a regular Curly inversion in both arms, others in only one arm (either 2L or 2R). One larva had not only the inversion showing in 2R, but also the translocated chromosome 4.

4. T2,4-A 1 R-2. The original non-Curly male was crossed to wild type virgins. Part of the resulting larvae showed both the 2L and 2R of the typical Curly inversions.

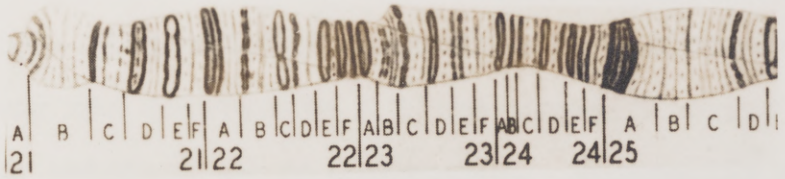
5. T2,4-A 45 R-2 males, heterozygous for the reversal, were crossed to wild type virgins. Some of the larvae from this cross showed the typical Curly inversions in both 2L and 2R, while others had the translocation figure, showing this to be a change in Curly rather than in the translocation.

6. T2,4-A 45 R-4 was treated in the same manner as the preceding case. Both 2L and 2R are typical Curly inversions, corresponding band for band in the salivary chromosomes. See figure 1 for an illustration of this case.

T.H.M.

Fig. 1. Typical Curly inversion figure.
(Case T2,4-A 45 R-4.)

a.



b.

c.

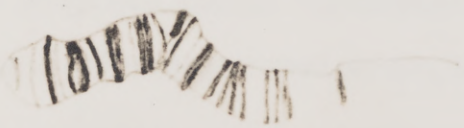


Fig. 2. Curly Reinversions.
a. Normal chromosome 2 (after Bridges);
b. lethal-1 R-3; c. lethal-7 R-4.

7. T2,4-A 45 R-5 was treated in the same manner as the preceding with similar results.

8. T2,4-A 45 R-7 was treated in the same manner as the other "45" reversals. It too is a reversal of Curly with no change apparent in the salivary chromosomes.

9. lethal-1 R-6. The original male of this reversal was crossed to wild type virgins. It was found that this case also is a reversal of the Curly character with no change in the Curly inversions.

10. lethal-7 R-4 was picked up in a non-Curly male which was crossed to wild virgins. The resulting larvae showed 2R to be the unaltered inversion, whereas there had been a reinversion of the 2L portion. This reinversion was not an exact one, since at the distal point of breakage of the original Cy 2L there was a slight bulge containing an extra band, with nothing comparable in its homologue. No difference was detectable a t the proximal point of break of the original Cy 2L inversion. Apparently one break took place at the exact point of the original distal break, while the other break took place about one band distal to the original proximal break.

11. lethal-1 R-3. Heterozygous non-Curly males were crossed to wild type virgin females. In this case some of the larvae showed the regular Curly inversion in 2R, but 2L has been reinverted. The reinversion in this case

is not exact, as can be seen from figure 2. As well as can be determined at the present time, one break occurred at the distal Curly 2L break, while the other took place approximately 4 bands proximal to the proximal Curly 2L break.

12. lethal-7 R-5. The original non-Curly male was crossed to wild type females, and in the resulting larvae several different figures were seen. Several appeared normal in 2L, but in 2R there was the Curly inversion. Others showed both arms of the second to have Curly inversion figures. Still other larvae showed perfectly normal appearing second chromosomes. The only explanation of this case seems to be that the original male was genetically mosaic. His offspring to date have not been checked to see if the chromosome with both arms inverted will produce flies phenotypically Curly or not.

C. A reversal of Curly from X-rayed females

T2,4-A 12 R-6 arose from a n X-rayed female. The original non-Curly male was crossed to wild type virgin females and the salivary chromosomes of the ensuing larvae examined. The right arm of the second chromosome presented the typical appearance of the Curly 2R, while the left arm was apparently normal. A close scrutiny of the latter in the regions of the original breaks showed the two homologues to be paired band for band. Quite obviously this is a complete and exact reinversion of the Curly 2L inversion, accompanied

by a reversal of the Curly phenotype. Apparently there has been no reversal of the lethal in this case, since none of the Curly offspring of this male show the recessive purple, this gene supposedly being present in all of the Curly chromosomes.

D. Point lethal reversals

1. lethal-1 R-1 arose in a non-Curly female, which was crossed to her brothers. Observations upon the salivary chromosomes were made on the F_1 larvae of this cross. Several such larvae showed the second chromosome to be perfectly normal throughout. Since only the lethal-1 chromosome in this stock was morphologically normal, this means that a reversal of the point lethal must have taken place.

2. lethal-1 R-2. In this case, as in the preceding, the cytological observations were made on the F_1 larvae from the original non-Curly female crossed to her brothers. Most of the larvae examined showed the typical Curly inversion, but one was normal throughout the entire second chromosome, showing this to be, like the preceding case, a reversal of the point lethal.

3. lethal-1 R-5. This case likewise came from a non-Curly female, and the cytological work was done on the F1 larvae, the fathers again being the lethal-1/Cy males. This case was checked very thoroughly in the salivary chromosomes and was found to be absolutely normal, band for band in both

2L and 2R, so that it too must be a reversal of the point lethal.

DISCUSSION OF RESULTS

One striking fact about the entire experiment is the large number of Curly reversals, the majority of which are essentially similar in their characteristics. These demonstrate repeatedly that the Curly character is separable from both the 2L and the 2R inversions, and therefore must be a point mutation,, rather than a position effect. Likewise, the lethal effect is independent of the phenotype, since at least in some of the cases a reversal of the latter effect does not affect the former. So far there is no proof that the lethal in the Curly chromosome has been altered. Further tests can be made on the various Curly reversals to see if all of the combinations are lethal.

A very interesting phase of these Curly reversals is the exact reinversion of the Curly 2L inversion, which took place as a result of X-raying the stock in the female rather than in the male. From a perusal of Grüneberg's (1937) work on the exact reinversion of the X chromosome inversion, roughest[3] with an accompanying reversal of the phenotype, the idea occurred that it might be possible to produce exact reinversions by means of X-raying females heterozygous for the inversion, for here the two points of original break would be in close approx-

imation at least during synapsis. This is apparently the case, since the only reversal picked up from the female was an exact reinversion. This might prove to be a good technique for analyzing supposed position effect mutations, since not one but both of the original breakage points can be broken again, and simultaneously. Presumably this should work as well with translocations as with inversions.

Reversals of the point lethal and of the translocation lethal show that at least some of the lethals are not deficiencies, since as Suche, et al (1938) point out, "Whatever is the nature of these lethal factors, they cannot be deficiencies unless one is willing to make the most improbable assumption that irradiation can create anew a particular gene or genes. They therefore must be point mutations or position effects, not losses, and these types of lethal mutations are capable of renewing their normal activity." It might be well to point out the fact that these point lethals were checked in the F_1 larva, so that there is no question of crossing-over or any other such possibilities.

In the light of this fact Demerec's standpoint that the great majority of lethals are deficiencies is untenable. While it is true that deficiencies are almost unanimously lethal, the opposite will not necessarily be the case, so

that even though certain abnormalities were obtained as lethals, one cannot draw far reaching conclusions from them.

It should be pointed out here that there is a possibility of difference between the lethals produced in one sex and the other from the standpoint of deficiencies and other rearrangements. The validity of this assumption is based on an experiment upon the relative rates of translocations in males and females (Parker, unpublished data). Using the multiple recessive method of Patterson, et al (1934) it was found that, while one out of every fifteen cultures derived from the male contains a 2-3 translocation, 502 F_2 cultures from irradiated females failed to produce any translocations of any type whatsoever. The two sexes were irradiated simultaneously, the dosage being around 2000 R units.

In this way it would seem that an experiment on the relative amounts of small chromosomal abnormalities in lethals produced in the male and in the female might prove interesting.

By no means is a consideration of the reversibility of lethals insignificant from the evolutionary standpoint. A change from the lethal to the normal, provided all lethals are not neomorphs and antimorphs, that is that they are hypomorphs and amorphs, would show that the assumption that lack of function is necessarily a loss is unsound, since these may revert and apparently reassume normal functioning.

SUMMARY

1. Stocks consisting of point lethals and 2-4 translocations balanced to Curly were X-rayed, and reversals were picked up in the form of non-Curly flies.

2. Cytological analyses of these cases show three types of reversals: reversals of the translocation-lethal, reversal of the Curly phenotype, and reversals of a point lethal.

3. It is pointed out that the Curly phenotype is independent of the Curly inversions and the Curly lethal.

4. Several reinversions of Curly 2L inversion are described, including an exact reinversion, which is accompanied by reversal of the Curly phenotype.

5. It is pointed out that the lethal mutations cannot be losses, since reversability is an adequate criterion of the presence of the gene or genes.

6. An attempt is made to point out the possible evolutionary significance of the reversability of lethals on the basis of the type of mutation involved, (i.e., hypomorphs, etc.)

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